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EXHIBIT H

A copy of pages 33 and 57-58 of the instant application, which provides support for performing site-directed mutagenesis.

ligating the ends together. See, generally, Sambrook et al., *supra*, and Glover and Hames, eds., *DNA Cloning*, 2d ed., Vols. 1-4, IRL Press, Oxford, 1995.

A recombinant nucleic acid molecule of the invention encoding a mutant FRIL family member can be prepared from wild-type DNA by site-directed mutagenesis (see, for example, Zoller and Smith, *Nucleic. Acids. Res.* 10:6487-6500, 1982; Zoller, M.J., *Methods Enzymol.* 100:468-500, 1983; Zoller, M.J., *DNA* 3(6):479-488, 1984.; and McPherson, M.J., ed., *Directed Mutagenesis: A Practical Approach*, IRL Press, Oxford, 1991.

A recombinant nucleic acid of the second aspect of the invention can be amplified by methods known in the art. One suitable method is the polymerase chain reaction (PCR) method described in Saiki et al., *Science* 239:487, 1988, Mullis et al., U.S. Patent No. 4,683,195, and Sambrook et al., *supra*. It is convenient to amplify the clones in the lambda-gt10 or lambda-gt11 vectors using lambda-gt10- or lambda-gt11-specific oligomers as the amplimers (available from Clontech, Palo Alto, CA). Larger synthetic nucleic acid structures can also be manufactured having specific and recognizable utilities according to the invention. For example, vectors (*e.g.*, recombinant expression vectors) are known which permit the incorporation of recombinant nucleic acids of interest for cloning and transformation of other cells. Thus, the invention further includes vectors (*e.g.*, plasmids, phages, and cosmids) which incorporate a nucleotide sequence of the invention, especially vectors which include the recombinant nucleic acid molecule of the invention for expression of a FRIL family member.

A recombinant nucleic acid of the invention can be replicated and used to express a FRIL family member following insertion into a wide variety of host cells in a wide variety of cloning and expression vectors. The host can be prokaryotic or eukaryotic. The nucleic acid can be obtained from natural sources and, optionally, modified. The genes can also be synthesized in whole or in part.

Cloning vectors can comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include plasmids from *E. coli*, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic

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 851  GCGTTCTGTG ATGATATATG TGTATCAATG ATTTTCTATG TTATAAGCAT
 901  GTAATGTGCG ATGAGTCAAT AATCACAAGT ACAGTGTAGT ACTTGTATGT
 951  TGTTTGTGTA AGAGTCAGTT TGCTTTTAAT AATAACAAGT GCAGTTAGTA
1001  CTTGT (SEQ ID NO: 3)
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A comparative illustration of the derived Dl-FRIL amino acid sequence with the reported amino acid sequence of the mannose lectin as determined by Gowda et al. (*J. Biol. Chem.* 269:18789-18793, 1994) is shown in Fig. 2. The single sequence derived for Dl-FRIL protein comprises domains that correspond directly and with substantial homology to the α subunit (SEQ ID NO: __) and β subunit (SEQ ID NO: __) of the protein described by Gowda et al., *supra*. When the β subunit of the Gowda et al. *(supra)* protein is assigned to the N-terminal domain and is followed linearly by the α subunit, the arrangement of the polypeptides shows homology to other legume lectins.

The derived Dl-FRIL amino acid sequence, however, comprises an additional of seven amino acid residues (aa27-34) that does not occur in the amino acid sequence described Gowda et al., *supra*. Several other differences between the amino acid sequences of Dl-FRIL and the amino acid sequence described by Gowda et al., *supra*, are also readily discernible from Fig. 2.

Site-Specific Mutagenesis

To establish functionality of homologs of the protein encoded by the Dl-FRIL cDNA, a mutation was made in the Dl-FRIL cDNA clone. The domains of the derived protein and the pea lectin that include the mutation site are shown below:

Dl-FRIL	.YLNPDYG.DPNYIHIGIDV	(SEQ ID NO: __)
Pea	FY.NAAWDPSNRDRHIGIDV	(SEQ ID NO: __)

It is known that the asparagine residue (the highlighted "N") in the pea lectin is involved in binding to its saccharide ligand. The corresponding asparagine in Dl-FRIL (position 141 of the amino acid sequence of SEQ ID NO: 2) was mutated to aspartic acid ("D"). This mutation was designated "N141D" for convenience.

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To introduce the mutation, recombinant PCR was performed (see, *e.g.*, Higuchi, R., *PCR Protocols: A Guide to Methods and Applications*, Innis M.A., Gelfand D.H., Sninsky J.J., and White TJ, eds., Academic Press, San Diego, 1990). Two PCR reactions were carried out separately on the full length cDNA using two primers that contain the same mutation and produce two products with an overlapping region:

MutI CCATAATCGGGATCAAGATAGGTG (SEQ ID NO: __)

MutII CACCTATCTTGATCCCGATTATGG (SEQ ID NO: __)

The primary PCR products were purified with the QIAquick PCR Purification kit (QIAGEN, Valencia, CA), according to the manufacturer's instructions. The overlapping primary products were then combined and amplified together in a single second reaction using flanking primers:

M1 Forw AACTCAGCCGCACAGTCATTGTCA (SEQ ID NO: __)

APEcoRI GAATTCGACCACGCGTATCGATGTCGAC (SEQ ID NO: __)

Both the primary and the secondary PCR reactions were performed in 100 μL containing 50 pmol of each primer, 0.4 mM deoxyribonucleotide and 1.0 unit Pfu polymerase (Stratagene) in the corresponding buffer. The primary PCR reaction amplified the two separate fragments in 30 cycles, each cycle comprising 40 seconds at 94°C, 40 seconds at 50°C, 60 seconds at 72°C, with an extension step at 72°C for 10 min. The second PCR reaction amplified the recombinant fragment in 12 cycles using the same conditions described above.

The resulting full-length fragment contained the mutation. The recombinant mutated product was cloned in the *Eco*RI site of the cloning vector pCR2.1, as illustrated schematically in Fig. 3, and sequenced as described above. This plasmid is referred to as "pCR2.1-DLA(D)."

Construction of Dl-FRIL-Expressing Plant Expression Vectors and *Nicotiana tabacum* Transformation

Recombinant PCR was used to modify the 5′ ends of both the wild-type and the mutant Dl-FRIL clones, to introduce a signal peptide for entry of the protein into the

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